

CLEAN VERSION FOR EXAMINER'S CONVENIENCE

1. [ORIGINAL] A process for detecting threonine or serine kinase activity in an immunoassay comprising the following steps:

a) providing a protein or peptide comprising the sequence motif

-Z-X-Y or -Y-X-Z-

wherein

Z = threonine or serine

X = a sequence of amino acids, preferably between 1 and 1000 amino acids, which may be the same or different

Y = tyrosine, threonine or serine

as a substrate for threonine or serine kinase, said protein or peptide being pre-phosphorylated at the Y position;

b) incubating the protein or peptide with a phosphate donor and a threonine or serine kinase to form a protein or peptide which is phosphorylated at postions Y and Z;

c) adding an antibody having a specificity to a peptide or protein which is phosphorylated at the Y and Z position; and

d) detecting the threonine or serine kinase activity.

2. [ORIGINAL] The process according to claim 1, wherein the phosphate donor is ATP, GTP, or a synthetic cosubstrate.

3. [ORIGINAL] The process according to claim 1, wherein the immunoassay is performed as a direct binding immunoassay, preferably a homogeneous direct binding immunoassay.

4. [ORIGINAL] The process according to claim 3, wherein a labelled peptide or protein is used as a substrate.

5. [ORIGINAL] The process according to claim 3, wherein a labelled antibody is used.

6. [ORIGINAL] The process according to claim 4, wherein the peptide/protein or antibody is labelled by a luminescent tag, a radioactive marker, a reporter enzyme or an affinity ligand.

7. [ORIGINAL] The process according to claim 1, wherein the immunoassay is performed as an indirect binding immunoassay, preferably a homogenous indirect binding immunoassay.
8. [ORIGINAL] The process according to claim 7, wherein a labelled ligand which is phosphorylated at its Y and Z position (bis-phosphorylated ligand) is added to compete with the protein or peptide which is phosphorylated at its Y and Z position (bis-phosphorylated ligand) for binding to the antibody.
9. [ORIGINAL] The process according to claim 8, wherein the bis-phosphorylated ligand is labelled by a luminescent tag, a radioactive marker, a reporter enzyme or an affinity legend.
10. [CURRENTLY AMENDED] The process according to claim 9, wherein the ligand comprises the amino acid sequence of SEQ ID NO:4 that is phosphorylated at amino acids 5 and 7, and particular is SEQ ID NO:4 that is phosphorylated at amino acids 5 and 7 and has 5-TAMRA-AEEA at the carboxy terminus.
11. [CURRENTLY AMENDED] The process according to claim 1, wherein the assay is performed as a fluorescence

immunoassay, in particular a fluorescence polarization immunoassay, a fluorescence correlation spectroscopic assay, a fluorescence resonance energy transfer assay, or a fluorescence intensity distribution assay.

12. [CURRENTLY AMENDED] The process according to claim 1, wherein X in the sequence motif comprises proline or glutamate or glycine.
13. [ORIGINAL] The process according to claim 1, wherein the protein provided is JNK1, JNK2 or JNK3 protein.
14. [ORIGINAL] The process according to claim 1, wherein the peptide provided includes sequences identical to those of JNK1, JNK2 or JNK3 active-site loop.
15. [CURRENTLY AMENDED] The process according to claim 1, wherein the peptide comprises the amino acid sequence of SEQ ID NO:2 wherein amino acid 5 is phosphorylated.
16. [CURRENTLY AMENDED] The process according to claim 1, wherein the peptide comprises the amino acid sequence of SEQ ID NO:1 wherein amino acid 7 is phosphorylated.
17. [ORIGINAL] The process according to claim 1, wherein the incubation of the protein or peptide is carried out in the presence of a threonine kinase.

18. [ORIGINAL] The process according to claim 17, wherein the threonine kinase is a mitogen-activated protein kinase kinase (MKK) (also named stress activated protein kinase kinase, SKK).
19. [ORIGINAL] The process according to claim 18, wherein the kinase is MKK7 (also named SKK4).
20. [ORIGINAL] The process according to claim 1, wherein the antibody is a monoclonal or polyclonal antibody.
21. [ORIGINAL] The process according to claim 20, wherein the antibody is a polyclonal antibody.
22. [ORIGINAL] The process according to claim 21, wherein the antibody is a polyclonal antibody specific for bis-phosphorylated, in particular active JNK.
23. [ORIGINAL] The process according to claims 1, wherein steps a) to d) are performed sequentially.
24. [ORIGINAL] A kit for detecting threonine or serine kinase activity in an immunoassay comprising the following components:
- a substrate as defined in claim 1;
 - an antibody as defined in claim 1; and

25. [ORIGINAL] The kit according to claim 24 further comprising a threonine or serine kinase, and/or reaction buffers including a phosphate donor, preferably ATP.
26. [ORIGINAL] The kit according to claim 24 further comprising a labelled ligand, preferably luminescently labelled ligand, said ligand comprising the following sequence motif

-Z-X-Y- or -Y-X-Z-

wherein

Z = threonine or serine

X = a sequence of amino acids, preferably between 1 and 1000 amino acids, which may be the same or different

Y = tyrosine, threonine or serine

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said protein or peptide ligand being phosphorylated at the Z and Y positions;

27. [ORIGINAL] A labelled ligand for use in a serine/threonine kinase assay comprising the sequence motif according to claim 26.

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28. [ORIGINAL] A use of the assay process according to claim 1, for screening modulators for threonine or serine kinase activity, in particular inhibitors for a threonine or serine kinase, or for detecting novel threonine or serine kinases.
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